Enzyme catalyzed oxidative cross-linking of feruloylated pectic polysaccharides from sugar beet - DTU Orbit (31/12/2018)

Enzyme catalyzed oxidative cross-linking of feruloylated pectic polysaccharides from sugar beet: Kinetics and rheology

Sugar beet pulp is a byproduct from sugar production consisting mainly of cellulose and pectic polysaccharide. Its utilization has been mostly as feedstock due to its high content of energy and fiber. This study emphasizes on the utilization of the pectin and arabinan fractions extracted from sugar beet pulp as a potential starting material for production of pectin derived products which could help maintain the competitiveness of the sugar beet based industry. The overall objective of this study has been focusing on understanding the kinetics of enzyme catalyzed oxidative cross-linking of feruloylated polysaccharide from sugar beet and relating the kinetics of this crosslinking to the properties of the cross-linked products. Several hypotheses have been formulated in order to accomplish our objective.

The first part of the study utilized arabinan-oligosaccharide fraction from sugar beet pulp byproduct. In this study we investigated the effect of arabianins backbone length on the kinetics of horseradish peroxidase (EC 1.11.1.7) (HRP) catalyzed oxidative cross-linking of ferulic acid (FA) moieties esterified to ?-(1,5)-linked arabinans; taking into account that FA can be oxidatively cross-linked by HRP catalysis in the presence of hydrogen peroxide (H2O2) to form ferulic acid dehydrodimers (diFAs). The composition of the substrate was analyzed by HPAEC, HPLC and MALDI-TOF, confirming the structural make up of the arabinan-oligosaccharide (Arabinose: 2.9- 3.4 mmol?g-1 DM; FA: 2.5-7.0 mg?g-1 DM) and verifying the formation of diFAs as a result of the enzyme catalyzed cross-linking reaction. The result demonstrates the influence of arabinans backbone length on the rates of FA cross-linking; longer arabinans exhibit a slower cross-linking rate than shorter, all other things being equal.

It has been our intention to study the rheological properties of cross-linked feruloylated arabinanoligosaccharide, however the attempt has not been fully achieved. It might be due to small molecular weight of the arabinan (?1.3 kDa) which prevented the measurement of the rheological properties since the change in viscosity resulting from the cross-linking was insignificant. Therefore, the next part of the work presented in this thesis utilized sugar beet pectin (SBP) solid fraction extracted from sugar beet pulp which has molecular weight >100 kDa. The compositional analysis of the substrate shows abundant amount of FA (7.3 mg?g-1 DM) in SBP which can be oxidatively cross-linked via enzyme catalyzed reaction by oxidoreductase enzymes. We hypothesized that different mechanisms of two oxidoreductase enzymes, i.e. HRP and laccase (EC 1.10.3.2), might influence the kinetics of the oxidative cross-linking and consequently the properties of the gels formed. The kinetics of oxidative gelation of SBP; taking place via enzyme catalyzed cross-linking of FA, was evaluated by small angle oscillatory measurements. The result indicates a significant difference between the SBP gels produced from the catalysis of HRP and laccase, that is, laccase catalysis produced stronger SBP gels albeit slower rates of gelation than the HRP catalysis. Statistically design experiment has been constructed to investigate the effect of several reaction factors which might influence the rates of gelation of SBP catalyzed by HRP or laccase, particularly the pectin level, temperature, enzyme dosage, pH and, for HRP, the H2O2 concentration. The result reveals that these reaction factors could be tuned in order to adjust the enzyme catalyzed gelation and the properties of the gels produced. Moreover, positive correlation between the rates of gelation and gel strengths was obtained for laccase catalyzed gels, but no such correlation exists for HRP catalyzed gels. Chemical analysis confirmed the formation of diFAs in the cross-linked products by both enzymes catalysis supporting that the gelation was a result of oxidative cross-linking of FA.

It is uncertain how the kinetics of enzyme catalyzed oxidative cross-linking of SBP and the gels properties are affected in emulsion systems. Thus, investigation on the enzyme catalyzed oxidative gelation of SBP was further performed on the SBP in emulsion systems. In this study, we have formulated two separate, identically composed, oil-in-water emulsion systems to study the effect of different methods of emulsion preparation on the emulsion stability in the presence of SBP and the kinetics of enzyme catalyzed oxidative gelation of SBP. The result shows that the different methods of emulsion preparation affect the emulsion stability and the rates of gelation of SBP in emulsion systems, and stronger gels were produced in the SBP containing emulsions as compared to the SBP without emulsions.

From this study, we have shown that arabinan-oligosaccharide and SBP solid fractions extracted from sugar beet pulp byproduct could undergo oxidative cross-linking of the feruloyl group, which abundantly esterified to the arabinan side-chains, through enzyme catalyzed reaction. Our study provides the insight into the relationship between the kinetics of the oxidative cross-linking of FA with the structural characteristic of the oligosaccharide, and the correlation between the rates of enzyme catalyzed oxidative gelation of feruloylated polysaccharide and the rheological properties of the gels produced. This knowledge could be useful for designing application of sugar beet pectin in food technology or similar application.

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